1015-Plat
Probing the Conformation Landscape of the Unfolded State: Do Disordered and Unfolded Dynamics Differ?
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Intrinsically disordered proteins undergo conformational changes that are often beyond the scope of current computational techniques used to study the dynamics of folded proteins. New approaches to define a metric for the dynamics of disordered proteins have been developed which are also readily applicable to the study of non-equilibrium globular protein dynamics. We use dimensionality reduction and clustering techniques applied to molecular dynamics (MD) simulations of a class of entirely disordered proteins (outside of a small anchoring domain) involved in nucleocytoplasmic transport, the FG-nucleoporins (FG-nups), as well as folding simulations of several globular proteins of similar size and sequence composition to compare disordered protein dynamics to early-stage folding dynamics. Our results provide detailed maps of the protein conformation space, allow us to classify proteins based on their dynamics, and indicate that disordered protein motion is of higher-dimensionality than early-stage folding dynamics.

1016-Plat
Generating Context-Specific Functions with Intrinsically Disordered Domains
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During animal development, Hox transcription factors specify many different tissues, including organs, appendages, and portions of structures that span the length of the animal, such as the nervous system, musculature, and ectoderm. Consequently, Hox proteins must sense the cellular identity and respond by instigating the appropriate gene regulatory program. To further complicate matters, Hox proteins interact with DNA via a homeodomain, which binds with high affinity yet notoriously low specificity. Using the *Drosophila* Hox protein Ultrabithorax (Ubx) as a model system, we have discovered that most of the Ubx sequences outside of the homeodomain are intrinsically disordered and regulate DNA binding. An intrinsically disordered region near the homeodomain modulates both DNA affinity and specificity. This region also mediates interactions with the Hox cofactor Extradenticle, and is alternatively spliced. Consequently, Extradenticle availability and alternative splicing may control DNA site selection. Ubx also must regulate genes in a position-specific manner within a single tissue. A second large intrinsically disordered domain modulates Ubx affinity and is required to bind transcription factors controlled by the WNT and TGFb/BMP cell signaling cascades. Since these signaling pathways subdivide Ubx-specified structures, this mechanism may control position-specific gene regulation within a field. Finally, once a Hox protein has bound DNA, it must select whether to activate or repress transcription. We find DNA binding by Ubx induces a conformational change, which increases the solvent exposure of the activation domain. We are currently investigating whether this conformational change allows DNA sequence to dictate the mode of transcription regulation. Using our carefully developed methods to generate and assay soluble, full-length *Drosophila melanogaster* Hox protein Ubx, we are identifying the mechanisms that drive context-specific Hox function in response to increasingly spatially restricted cues (tissue identity, location within a tissue, and DNA binding sequence).

1017-Plat
The Solution and Binding Behavior of the Intrinsically Disordered FG Nups Determined by STINT-NMR
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The Nuclear Pore Complex (NPC) mediates all transport between the nucleus and cytoplasm. The channel of the NPC is lined with ''FG Nups'', a family of intrinsically disordered proteins characterized by phenylalanine-glycine repeat motifs. FG nups form the exquisitely selective filter of the NPC; non-binding proteins are excluded while the binding of transport factors to the FG Nups facilitates their passage through the NPC. Like other intrinsically disordered proteins, the FG Nups appear to be very sensitive to their environment, showing vastly different behavior in different experimental conditions; in vitro, the observed behavior of the FG Nups varies from rigid gels to flexible random-coil polymers. We used STINT-NMR to probe the behavior of a model FG Nup within a living cellular environment. In a STINT-NMR experiment, NMR observations are directly performed on bacteria in vivo co-expressing a labeled protein and an unlabeled binding partner. We have found that the solution state of the FG Nup within living cells is completely disordered, while NMR spectra are significantly changed in vitro buffers, presumably from numerous intra- or inter-molecular contacts. Moreover, the binding interface between transport factors and the FG Nup differs considerably between solution and cellular conditions. Thus, a key determinant to FG Nup behavior is the local environment.
These results indicate that the proper behavior of the FG Nups is dependent on the normal cellular milieu, and is not necessarily represented in vitro; our findings have important implications for the various current models regarding the molecular mechanisms of nucleocytoplasmic transport and behavior of weak cellular interactions generally.
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PLATFORM Y: Membrane Fusion

1018-Plat
Visualizing Release of Single Fluorophores at Membrane Fusion Sites
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SNARE proteins play a central role in nearly all intracellular fusion reactions; fusion is driven by formation of trans-SNARE complexes (SNAREpins) through pairing of vesicle-associated v-SNAREs with complementary t-SNAREs on target membranes. We recently reported a docking and fusion assay in which single small unilamellar vesicles containing the synaptic/exocytic v-SNAREs VAMP/synaptobrevin (v-SUVs) fuse rapidly with planar, supported bilayers containing the synaptic/exocytic t-SNARE syntaxin-SNAP25 (t-SBLs), with single fusion events occurring in ~130 ms after docking. We optimized acquisition conditions such that now the release of single fluorescently labeled lipids can be visualized. As the fluorophores diffuse away from the release site, individual spots become discernible and can be tracked with ~16 ms resolution. More than 90% of the tracked spots bleach in a single step, strongly suggesting they represent single fluorescent lipid molecules. Intensity-drops as spots disappear follow a normal distribution whose mean defines the intensity of a single fluorophore. This allows us to estimate the total number of lipids in a vesicle prior to its fusion with the t-SBL, given the label density. An independent estimate of vesicle size is based on extrapolating the number of surviving spots as a function of time to just before fusion.
We studied the effects of lipid composition. In bilayers containing nearly physiological amounts of cholesterol, the mean squared displacement of single fluorescent lipids that are released into the t-SBL increases linearly in time indicating a diffusive process and yields a diffusion coefficient of ~0.3 $\mu m^2/s$, a value that is similar to those found for lipid diffusivities in the plasma membrane of live cells. In addition, the majority of the vesicles that dock end up fusing. In contrast, in bilayers devoid of cholesterol, lipid diffusion is much faster, but only 40-50% of docked vesicles fuse.

1019-Plat
Synaptotagmin Expands Membrane Fusion Pore by Facilitating SNARE-Complex Formation
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Pore expansion, an essential step for SNARE-mediated membrane fusion, has not been well studied due to the lack of a reliable content mixing assay. Recently, we have developed a new assay to detect the inter-vesicular mixing of large cargoes at the size of several nanometers at the single molecule and vesicle level [1]. Through our new assay, we found that the neuronal SNARE complexes alone, without regulatory proteins, are able to expand fusion pore inefficiently. By interacting with t-SNARE proteins through a *trans*-conformer [2], membrane anchor synaptotagmin 1 and Ca^{2+} facilitate SNARE complex formation for fusion pore expansion.
References
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1020-Plat
Extracellular Ca^{2+} Directly Inhibits Exocytosis in Neurons
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Exocytosis of transmitter releasing vesicles is elicited by an elevation of *intracellular* Ca^{2+} concentration ($[Ca^{2+}]_i$). Given the existing Ca^{2+} sensor receptor (CaSR), although $[Ca^{2+}]_i$-induced exocytosis is fully established, however, whether *extracellular* Ca^{2+} (concentration $[Ca^{2+}]_o$) directly regulates exocytosis is not rigorously examined yet. Here we report that extracellular Ca^{2+} inhibited exocytosis following moderate $[Ca^{2+}]_i$ rises (2-3 μM), which were triggered by either photolysis of caged Ca^{2+} or caffeine. IC_{50} of extracellular Ca^{2+} inhibition on exocytosis (ECIE) was 1.38 mM, so that a physiological reduction (~30%) of $[Ca^{2+}]_o$ significantly increased the evoked exocytosis. On single vesicle level, the quantal size and release frequency were significantly regulated by physiological $[Ca^{2+}]_o$. Involvement of CaSR in ECIE was excluded by experiments of pharmacology and molecular biology. Thus, as an extension of the classic Ca^{2+} hypothesis of synaptic release, physiological extracellular Ca^{2+} plays dual roles in evoked exocytosis by providing source of Ca^{2+} influx, and by directly regulating quantal size and release probability in neuronal cells.

1021-Plat
Strong, Positive Cooperativity of SNARES For Fusion Pore Opening Studied At the Single-Molecule Level
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Single vesicle fluorescence assay detects the fluorescence signals from surface-immobilized nano-scale vesicles (typically diameter of 50 nm). There are three principal labeling positions: vesicle membranes, luminal contents and membrane proteins, each of which allows for study of different aspects of membrane-related biological processes. During the past five years, we have reported two realizations of these possibilities: Measuring the kinetics of single vesicle-vesicle fusion by labeling vesicle membranes [1-3] and detecting fusion pore opening in such single vesicle fusion by encapsulating fluorescently-labeled DNA hairpins inside vesicles [4]. In this work, by using fluorescently labeled SNARE proteins, we report on the kinetics of SNARE complex formation observed at the single-molecule level. For this purpose, we have developed an advanced single-molecule FRET technique, in which we track not one, but up to 10 proteins at the same time while keeping the precision at the single-molecule level. The measured kinetics of SNARE complex formation shows strong, positive cooperativity. We finally discuss whether we can make two single-molecule measurements in one experimental setting, for example, detecting the moment of fusion pore opening while tracking formation of multiple SNARE complexes. Such experiment would reveal quantitative correlation between multimeric structure of SNARE proteins and its functional effect on fusion pore opening.
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1022-Plat
HIV Fusion Peptide Penetrates, Disorders and Softens T-Cell Membrane Mimics
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This work investigates the interaction of N-terminal gp41 fusion peptide (FP) of HIV-1 virus with model membranes in order to elucidate how FP leads to fusion of HIV and T-cell membranes. FP constructs were (i) wildtype FP23 (23 N-terminal amino acids of gp41), (ii) water soluble monomeric FP that adds six lysines on the C-terminus of FP23 (FPwsm) and (iii) the C-terminus covalently linked trimeric version (FPtri) of FPwsm. Model membranes were (i) LM3 (a T-cell mimic), (ii) DOPC, (iii) DOPC/$30_{mole\%}$cholesterol, (iv) diC22:1PC and (v) diC22:1PC/ $30_{mole\%}$cholesterol. Diffuse synchrotron low-angle x-ray scattering (LAXS) from fully hydrated samples, supplemented by volumetric data, showed that FP23 and FPtri penetrate into the hydrocarbon region and cause membranes to thin. Depth of penetration appears to depend upon a complex combination of factors including bilayer thickness, presence of cholesterol and electrostatics. X-ray data showed an increase in curvature in hexagonal phase DOPE which further indicates that FP23 penetrates into the hydrocarbon region rather than residing in the interfacial headgroup region. LAXS data also yielded bending moduli K_C, a measure of membrane stiffness, and wide-angle x-ray scattering (WAXS) yielded the S_{xray} orientational order parameter. Both FP23 and FPtri decreased K_C and S_{xray} considerably, while the weak effect of FPwsm suggests that it did not partition strongly into LM3 model membranes. Our results are consistent with the HIV fusion peptide disordering and softening the T-cell membrane, thereby lowering the activation energy for viral membrane fusion. This research was supported by NIH Grant GM 44976 (STN,RC,JFN) and NIH AI 47153(WQ,DPW).

1023-Plat
PE Lipids in Single SNARE Vesicle Fusion Assay on Supported Membranes Promote Docking and Reduce the Number of SNARE Complexes Required for Fast Fusion
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SNARE proteins are the core of the membrane fusion machinery in mammalian cells. Both in vitro and in vivo studies focus mostly on neuronal SNAREs (syntaxin1A, SNAP25, synaptobrevin2), which are involved in Ca2+ regulated exocytosis of synaptic vesicles. A recently developed single vesicle SNARE-mediated fusion assay in planar supported bilayers allows us to study individual docking and fusion events with a millisecond time resolution in a well-controlled lipid environment. Although, the fusion time in this assay is differently defined than in cellular settings, various parameters describing SNARE-mediated membrane fusion can be obtained, including the efficiency of vesicle docking and the probability of fusion after docking. Additionally, the number of particles in the fusion site and their activation rates can be determined from modeling of the fusion kinetics data.
To better mimic the composition of the plasma membrane, we added various concentrations of PE and PS to our standard PC/Chol mixture. We observed a 1.5- to 3-fold increase in docking efficiency when the PE content was raised from 0 to 30%. In contrast, the fusion probability decreased 3- to 6-fold compared to PC/Chol membranes. Interestingly, including PE only in the vesicle membrane was sufficient to cause these effects. A detailed analysis of the fusion kinetics revealed that the fusion rate did not change significantly while the number of SNARE complexes that drive fast membrane fusion decreased with 3 complexes constituting the minimal fusion site. These results are rationalized with a model, in which fewer SNAREs are required to overcome the lower activation energy barrier in the presence of negative curvature-promoting PE than the higher activation energy that is likely present in the absence of PE.

1024-Plat
The Pathway to Fusion in Synthetic Membrane Systems
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Considerable evidence indicates the pathway to fusion of synthetic and biological membranes consists of two stages. The first is a transition to a hemifused intermediate where only contacting leaflets are fused and distal leaflets engaged in a hemifusion diaphragm (HD). The second consists in rupture of the growing or fully developed HD to give fusion. Here we present a quantitative model for this pathway in protein-free systems. The first stage is described by our model of hemifusion (presented elsewhere) which outputs time-evolutions of HD tension and area. We find that complete fusion results only if the HD tension, initially twice that outside the HD, excedes the lysis threshold for sufficiently long. Fusion does not occur instantaneously after hemifusion nucleation since membranes transiently tolerate tensions above lysis as previously demonstrated by micropipet aspiration of vesicles [Evans et al, *Biophys J*, 2003]. Tension relaxes rapidly with HD size such that a growing HD surviving the initial high tension episode matures into a dead-end hemifused state. Thus fusion and dead-end hemifusion are alternative outcomes; the favored outcome depends critically on cation concentration, lipid composition and other physical factors. We find higher salt levels create higher tensions which favor complete fusion. Predicted fusion times are in quantitative agreement with a study of giant vesicles where HD rupture yielded fusion after ~1-2 s in 6 mM Ca^{2+} [Nikolaus et al, *Biophys J*, 2010]. We predict dead-end hemifusion at lower salt, consistent with stable HDs reported in 2 mM Mg^{2+}. In suspended bilayer-vesicle systems we find cation-induced vesicle tensions are dissipated upon hemifusion and thus Ca^{2+} drives dead-end hemifusion only. This is in agreement with the consistently reported experimental finding that calcium alone cannot fuse vesicles to bilayers, and fusion requires additional osmotic swelling.